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IHR ZEICHEN / YOUR REF.
PCT/EP03/13655

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Application/Patent No:
PCT/EP03/13655

Applicant/Proprietor:
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Encl
Computer disc containing sequence listing; paper copy; amended description page 52.

This is in response to the official communication dated 11.03.2004.

Applicant hereby submits a sequence listing in computer-readable form according to WIPO Standard St.25, as well as in the form of a paper copy.

It is confirmed that the sequence information recorded on the data carrier is identical to the written sequence listing. It is further stated that the sequence listing does not include subject matter which goes beyond the content of the application as originally filed.

Applicant also submits herewith amended

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description page 52 wherein the obvious error in the incorrect sequence listing reference in line 15 „SEQ ID NO. 14" has been corrected to read „SEQ ID NO. 26".

M. Dey

Dr M Dey

Taken together the over-expression of Prl-1 showed an effect on metabolism of exogenous free fatty acids and glucose as well as triglyceride storage in all three assays we performed in SGBS cells, making it a potential interesting drug target for the treatment of diabetes and related metabolic disorders.

Example 6: Assays for the determination of lipid storage, synthesis and transport of Prl-1 LOF adipocytes (Figure 6)

Loss of function in 3T3-L1 adipocytes by RNAi technique

In order to stably inhibit Prl-1 expression, 3T3-L1 preadipocytes were engineered by retroviral infection aimed to express a target specific short interfering RNA construct under the control of the human hH1 promoter according to Brummelkamp et al. (Science 2002, Vol 296, p. 550-553). The following Prl-1 specific RNAi sequence was used: AGG ATT CCA ATG GTC ATA G (SEQ ID NO. 26).

Retroviral infection of preadipocytes

Packaging cells were transfected with a retroviral plasmid pLPCX carrying the specific RNAi construct under the control of the human hH1 promoter and a selection marker using calcium phosphate procedure. Control cells were infected with the same vector carrying no transgene.

Briefly, exponentially growing packaging cells were seeded at a density of 350,000 cells per 6-well in 2 ml DMEM + 10 % FCS one day before transfection. 10 min before transfection chloroquine was added directly to the overlying medium (25 µM final concentration). A 250 µl transfection mix consisting of 5 µg plasmid-DNA (candidate:helper-virus in a 1:1 ratio) and 250 mM $CaCl_2$ was prepared in a 15 ml plastic tube. The same volume of 2 x HBS (280 µM NaCl, 50 µM HEPES, 1.5 mM Na_2HPO_4, pH 7.06) was added and air bubbles were injected into the mixture for 15 sec. The transfection mix was added drop wise to the packaging cells, distributed and the cells were incubated at 37°C, 5 % CO_2 for 6 hours. The cells were washed with PBS and the medium was exchanged with 2 ml DMEM + 10 % CS per 6-well. One day after transfection the cells were washed again and incubated for 2 days of virus collection in 1 ml DMEM + 10 % CS per 6-well at 32°C, 5 % CO_2. The supernatant was then filtered through a 0.45 µm cellulose acetate filter and